Effects of vascular normalization on progression of prostate cancer and therapeutic efficacy

You Hyun Kang

Department of Medical Science

The Graduate School, Yonsei University

Effects of vascular normalization on progression of prostate cancer and therapeutic efficacy

Directed by Professor Sung Joon Hong

The Master's Thesis
submitted to the Department of Medical Science
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

You Hyun Kang

June 2016

This certifies that the Master's Thesis of
You Hyun Kang is approved.

Thesis Supervisor : Sung Joon Hong

Thesis Committee Member : Kyung Seok Han

Thesis Committee Member : Nam Hoon Cho

The Graduate School
Yonsei University

June 2016

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

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You Hyun Kang

Department of Medical Science
The Graduate School, Yonsei University

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Targeting VEGF pathway suppresses tumor progression by starving tumor cells though blocking tumor blood supply but hypoxia induced by blocking vessels aggravates tumor progression and induces metastatic potential and treatment resistance. Tumor vessels are typically leaky, dilated, Saccular, and tortuous. These abnormal blood vessels result in a hostile tumor microenvironment characterized by hypoxia, low pH and high interstitial fluid pressure. Impaired blood flow interferes drug delivery to solid tumor and impedes therapeutic efficacy of cancer agents. Here, we investigated role of vascular normalization in cancer progression and delivery of chemotherapeutic agent in prostate cancer. Normalization of vasculature by reducing vascular leakiness reduced hypoxic phenotype and improved vascular perfusion in the PC-3 xenografts. It also increased concentration of chemotherapeutic agent. Our study demonstrated that direct normalization of vasculature by vascular normalizing agent could provide a strategy to prostate cancer and the efficacy of chemotherapy.

Key words : vascular normalization, tumor angiogenesis, docetaxel, prostate cancer

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I. INTRODUCTION

Tumors acquire blood supply via multiple mechanisms including angiogenesis by sprouting new vessels from existing vessels, cooption, intussusception, vasculogenesis, vascular mimicry, and trans-differentiation of cancer cells into endothelial cell.[1,2] Anti-angiogenic therapies have been used as anti-cancer therapies in various type of cancer.[3] One of the first clinically available anti-angiogenic drugs bevacizumab, which is a recombinant humanized monoclonal antibody targeting VEGF-A.[4]

Targeting VEGF pathway suppresses tumor progression by starving tumor cells though blocking tumor blood supply. However, several studies showed that hypoxia induced by blocking tumor vessels aggravates tumor progression and induces metastatic potential and treatment resistance.

Tumors commonly exhibit an abnormally thick basement membrane and perivascular cells with abnormal morphology.[3] Although the extent and abnormality varies with tumor type and location, tumor vessels are typically leaky, dilated, saccular, and tortuous. These abnormal blood vessels result in a hostile tumor microenvironment characterized by hypoxia, low pH and high interstitial fluid pressure. These hypoxic and acidic stresses alter the intrinsic characteristics of tumor cells and select more aggressive and metastatic clones. Moreover, hypoxia upregulates the production of angiogenic factors by cancer

and stromal cells, which further aggravate vessel disorganization and thereby fuel non-productive angiogenesis in an endless self-reinforcing loop.[5] Impaired blood flow interferes drug delivery to solid tumor and impedes therapeutic efficacy of cancer agents.

Negative effects of abnormal vasculatures in tumor microenvironment on drug delivery and its improvement after vascular normalization has been investigated. Yuan F et al. investigated time-dependent vascular regression by an anti-vascular endothelial growth factor (VEGF) antibody and suggested that decreased vascular permeability after anti-VEGF antibody increase concentrations of drugs and oxygen in human tumor xenografts.[6] This study showed that first evidence of drug-induced normalization of tumor blood vessels. Normalization of blood vessels could improve the availability of drugs and oxygen to disease areas, and thus improve combination therapy.[7] More recent studies using genetically modified mice showed that normalization of blood vessels improved tumor perfusion and oxygenation and inhibited tumor cell invasion and metastasis and also the therapeutic efficacy of chemotherapies and immunotherapies improved.[8-10] These results suggest that chemotherapy after normalizing by repairing the function of tumor vessels may be a promising strategy to slow tumor progression and enhance cancer treatment.

Docetaxel was used as a monotherapeutic and in combination with other therapeutic. Breast, prostate, and non-small cell lung cancer was used docetaxel for therapeutic agent.[11-15] Especially, docetaxel is an anticancer drug that is commonly used in prostate cancer. Therefore, we investigated role of vascular normalization in cancer progression and delivery of chemotherapeutic agent in prostate cancer. Here, our study demonstrates that direct normalization of vasculature by vascular normalizing agent could provide a strategy to prostate cancer and the efficacy of chemotherapy.

II. MATERIALS AND METHODS

1. Cell culture

Human Umbilical Vein ECs (HUVECs) were isolated from human umbilical cord veins by collagenase treatment. Cells were grown in 2% gelatin coated dishes and maintain in M199 (Hyclone, Thermo Scientific, Canada) supplemented with 20% fetal bovine serum (FBS ; Hyclone), 1% penicillin/streptomycin (Thermo Scientific), 3 ng/ml basic fibroblast growth factor (R&D system), and 5 U/ml heparin(Sigma) at 37℃ in a humidified 95–5% (v/v) mixture of air and CO2. PC-3 and LNCaP cells were maintained in the RPMI 1640 (Hyclone) supplemented with 10% FBS and penicillin–streptomycin (Thermo Scientific). Cells were grown at 37°C with 5% CO2.

2. Drug

Sac-1004 (Leakage Blocker 1004) was purchased from Sigma-Aldrich. Working solution of Sac-1004 is prepared by diluting stock (in dimethyl sulfoxide; DMSO) in phosphate buffered saline (PBS). Docetaxel and Paclitaxel were kindly donated by Severance Cancer Hospital, Korea.

3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

HUVECs were seeded at a density of 3 x 104 cells/well in gelatin-coated 24-well plates and incubated for 24 hr. Cells were washed and switched to serum free media and treated with various concentrations of Sac-1004. After 48 hr cells were washed and serum free media containing MTT (0.1 mg/ml) was added followed by incubation at 37℃ for 3 hr. The residual MTT was carefully removed and the crystals were dissolved by incubation with DMSO. The absorbance was measured at 570 nm spectrophotometry.

4. Immunofluorescence

HUVECs and PC-3s were subjected to immunostaining. Briefly, the cells

were fixed in 4% formaldehyde for 10 min at 4 ℃. After fixation, the cells were permeabilized in 0.1% Triton X-100 in PBS for 10 min at 4 ℃. Cells were incubated for overnight at 4 ℃ with antibodies such as anti-VE-cadherin (1:100, Santa Cruz, CA), CD31 (1:100, Abcam, UK). The cells were incubated with secondary antibody conjugated with Alexa for 1 h at room temperature. Cells were mounted using DAKO mounting (Sigma) reagent and are observed using a fluorescence microscope (Zeiss; 400x, Oberkochen, German).

5. Tumor model and treatment regime

Tumors were subcutaneously established by injecting PC-3 cells (5 x 10^5 cells; 100μl) on the lateral flank of 7-week-old BALB/C nude mice. Sac-1004 (50mg/kg) was administered intravenously daily for 7 days or every week for 4 wk. Mice were received combination therapy or Docetaxel alone intravenously injected with Docetaxel (20mg/kg) every week for 4 wk. Tumor volumes measurement was daily with calipers and calculated as (width x length x height) / 2. Tumors were captured after one week, one month.

6. Tumor permeability and vascular perfusion

Evans blue (Sigma) and fluorescein isothiocyanate (FITC)-dextran (40-kDa; Sigma) was used to identify tumor vascular leakage. Evans blue dye was injected intravenously 30 min prior to tumor capture. The tumor tissues were dried at 50 ℃ for overnight and the dye was extracted with 1 ml of formamide at 50 ℃ for overnight. Absorbance was taken at 620 nm. For FITC-dextran-mediated leakage assessment, an intravenous injection of 3 mg/mouse FITC-dextran was made 10 min before capture of tumor. Tumors were embedded in optimal cutting temperature (OCT) compound. Sections of 30 μm thickness were then directly viewed under fluorescence microscope to identify vascular leakage. Vessel perfusion was quantified using biotinylated lycopersicon esculentum lectin (Vector Laboratories, California, USA) injected intravenously 10 min before excision of tumor.

7. Histology and immunostaining

To evaluate tumor histology, tumors were captured and embedded in optimal cutting temperature compound and sectioned 30 μm thickness. Staining was performed by incubating with one of the following antibodies: VE-cadherin (1:100, Santa Cruz, CA), anti-CD31 (1:100, Abcam, UK). The sections were then incubated in Alexa Fluor-conjugated secondary antibodies.

Hypoxia was detected by pimonidazole (Hypoxyprobe-1; Chemicon, Burlington, USA) adduct formation caused by an intravenous injection of 75mg/kg pimonidazole 1 hr before capture of tumor. Adducts were stained with a monoclonal antibody directed against pimonidazole.

8. Contrast-Magnetic Resonance images (DC-MR imaging) and analysis

Magnetic resonance images were acquired using a Biospec 94/20 USR (Bruker, Germany). For each tumor, perfusion and vascular permeability were measured using a bolus intravenous injection of 0.2 mmol ⁄ kg DOTAREM (Guerbet, USA) mixed with saline. The MRI speed was measured for both the control and Sac-1004 treatments groups. Qualitative analysis was relied on an evaluation of the signal intensity curve behavior in the voxel or ROI.

9. Identification of docetaxel in vivo

The liver and tumor sample were collected 1 hour after docetaxel after i.v. infusion of docetaxel at a dose of 20mg/kg with or without sac-1004 at a dose at a 50mg/kg in mice. And rinsed with ice-cold 0.9% injectable NaCl solution and blotted dry with paper tissue. Liver and tumor sample were homogenized (IKA-Labortechnik, Staufen, Germany) in 3 volumes cold 0.9% injectable NaCl solution at 4 ºC. Then the homogenate suspension was centrifuged at 1000 rpm for 5 min at 4 ºC. All samples were kept at 4 ºC throughout the study.

10. Docetaxel liquid chromatograpy-tandem mass spectrometry

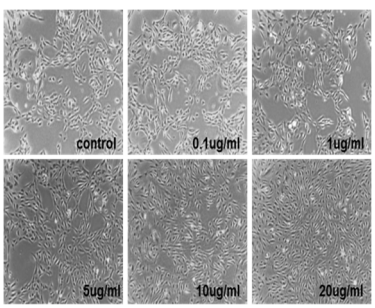
The Docetaxel and Paclitaxel (internal standard) were analyzed using an API 5500 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA), coupled with an Agilent 1260 HPLC system (Agilent Technologies, Wilmington, DE). The instrument was operated under positive [M + H]+ electrospray ionization and multiple reaction monitoring (MRM) modes. The optimized ion spray voltage was set at 5500 V for positive ionization mode, and temperature was 500 °C. Nitrogen gas used as the Ion source gas 1, 2 and curtain gas was set at 70, 10, and 10 psi, respectively. Sample separation was performed on an Agilent Poroshell 120 EC-C18 reversed-phase column (50 × 4.6 mm i.d., 2.7μm particle size). The mobile phase consisted of distilled water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) (10:90, v/v) at a flow rate of 0.4 mL/min. The total run time was 3.0 min per sample and the temperature of the autosampler was set at 4°C throughout the analyses. Data analyses were completed on Analyst®, version 1.5.2 software. (Sierra Analystics, LLC). An aliquot of 5μL supernatant was injected on to LC-MS/MS system for quantitative analysis.

III. RESULTS

1. Effects of an endothelial cell stabilizer on endothelial cells and prostate cancer cells

We initially investigated the effect of Sac-1004 on HUVEC. We found that Sac-1004 was able to enhance proliferation of HUVEC (Fig. 1A and B). In addition, we investigated the restores effect of sac-1004 on endothelial barrier integrity. VEGF is a signal protein produced by cells that stimulates angiogenesis therefore it is one of the molecules causing leakiness of tumor vessels. So, we induced the VEGF-mediated stress fiber formation. Immunohistochemical analysis of PC-3 tumor sections showed that sac-1004 treatment group had increased number of homogenous vasculature by restoring the linear distribution of the VE-Cadherin (Fig. 1C). VEGF treat group had leaky, irregular vasculature (white narrow) but, sac-1004 treat group had changed homogenously (yellow narrow). In other words, we found that sac-1004 treatment restored the disruption of VEGF-induced leakage by restoring the linear distribution of the VE-Cadherin. To confirm the viability effects of sac-1004 in prostate cancer cells, we experimented in the same way as previously HUVECs. sac-1004 had not cytotoxic effects by itself on prostate cancer cells in vitro (Fig. 1D and E). These results suggest that sac-1004 can reduce tumor vascular leakage without influencing tumor cell survival.

A



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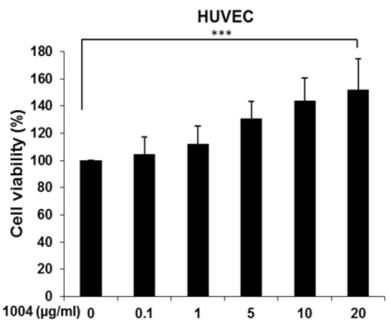

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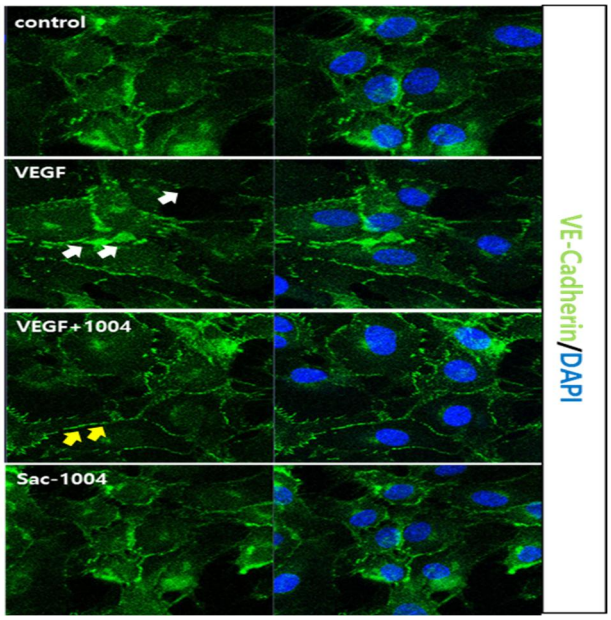

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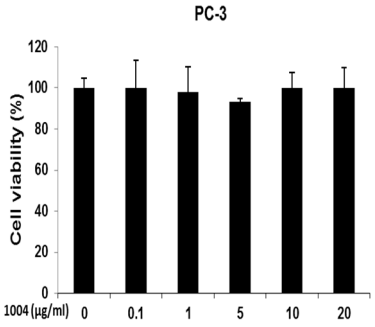

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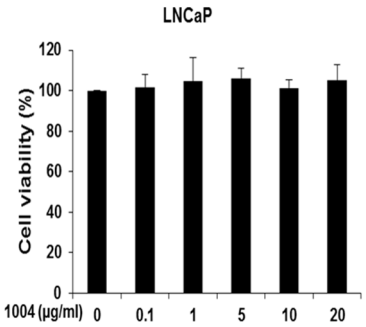

Figure 1. Effects of Sac-1004 on human endothelial cells and prostate cancer cells. (A) and (B). HUVECs were treated with Sac-1004 at different doses and cell proliferation was evaluated by MTS assay. (C). HUVECs were treated with Sac-1004 (10μg/ml, 3hr) followed by stimulation with VEGF (50ng/ml, 1hr). The representative images of HUVECs were taken by a light microscope (x magnification). (D) and (E). PC-3 cells, LNCaP cells were treated with Sac-1004 at different doses and cell proliferation was evaluated by MTS assay. Data are represented as mean ± S.D; ***P<0.001.

2. Effects of an endothelial cell stabilizer on vascular functions in prostate cancer

We showed that Sac-1004 was able to reduce tumor vascular leakage *in vitro*. So, We investigated the effect of Sac-1004 in PC-3 xenografts. We subcutaneously injected the PC-3 cells to mice and PC-3 tumors are treated with Sac-1004. Since sac-1004 had exhibited that it is possible to decrease tumor vascular leakage, we examined vascular leakage using Evans blue dye and FITC-dextran *in vivo*. Extravasation of Evans Blue in sac-1004 treated tumors had decreased as compared with control tumors. Namely, sac-1004 treated tumors had decreased ratio of leaky blood vessels as compared with control tumors (Fig. 2A).

FITC-dextran imaging reconfirmed visually as quantitative analysis. It indicates exudation of FITC-dextran from leaky vessels in control group. These results demonstrate that vascular-leakage blocking molecule sac-1004 which significantly reduced tumor vessel leakiness (Fig. 2B and C). Analysis of end-point tumor volume indicated that tumor growth was not affected in sac-1004 treatment. It confirmed that sac-1004 did not effect on tumor cell growth in a short period of time. (Fig. 2D).

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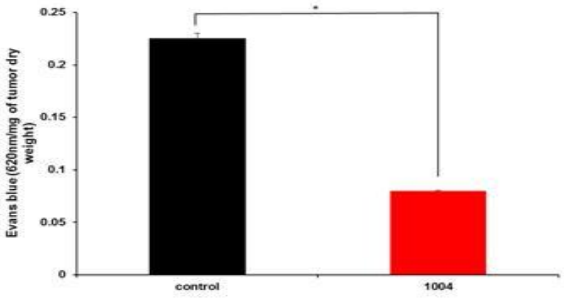
A



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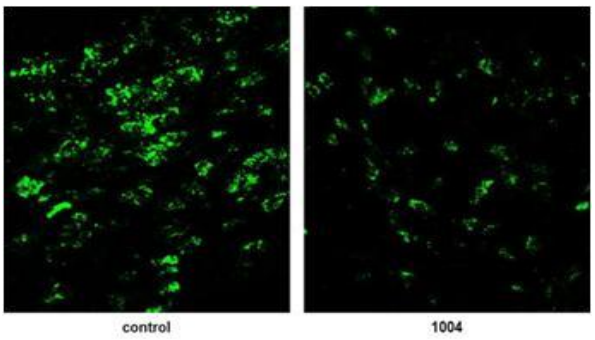

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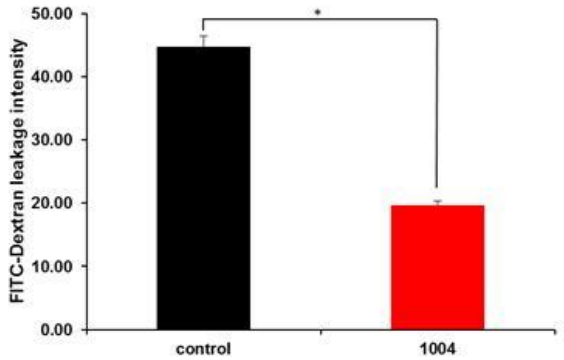

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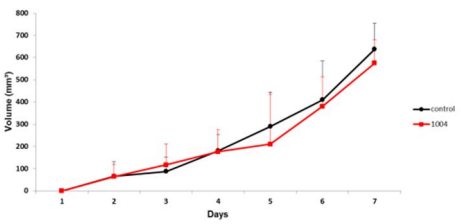

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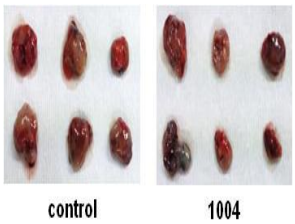

Figure 2. Sac-1004 did not affect on PC-3 tumor growth but it can reduce tumor vessel leakiness. (A). Assessment of vascular permeability using Evans blue extracted from the PC-3 xenografts tumors after the intravenous injection of Evans blue (n=6). (B). Tumor vascular leakage was visualized using FITC-dextran. Representative pictures have been shown for comparison (n=6). (C). Pictures shown in B. were quantified using ImageJ software. Data are represented as mean ± S.D; *P<0.05. (D). Tumor growth of PC-3 xenografts was measured every day during the treatment of sac-1004 (50mg/kg). (E). Pictures of PC-3 xenografts after Sac-1004 and control were shown.

3. Effect of vascular normalization on tumor hypoxia in prostate cancer

Previous studies have suggested that sac-1004 significantly reduced tumor vessel leakiness by restoring endothelial junction *in vitro*. Enhanced endothelial junction improves vascular structure and function. We conducted Immunohistochemical analysis whether sac-1004 can restore the endothelial junction. Immunohistochemical analysis of PC-3 tumors showed that increased number of VE-cadherin positive vessels were observed in PC-3 tumors treated with sac-1004 (Fig. 3A and B). In addition, control groups are discontinuous VE-cadherin lining but sac-1004 groups are continuous VE-cadherin lining. These results confirm that sac-1004 reduces tumor vascular leakage in vivo by stabilizing junction protein VE-cadherin. Improved vascular function can help alleviate hypoxic microenvironment. Therefore, we experiment hypoxia in sac-1004 treatment receiving tumors via pimonidazole staining. Hypoxia was detected by pimonidazole adduct formation caused by an intravenous injection of pimonidazole. Sac-1004 treatment significantly reduced hypoxia in PC-3 tumors (Fig. 3C and D). These data suggests that normalizing the tumor microenvironment by repairing the structure of tumor vessels may be a promising strategy to enhance cancer treatment like radiotherapy, chemotherapy, and immunotherapy.

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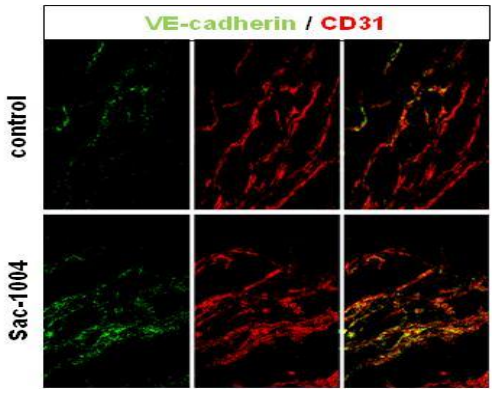

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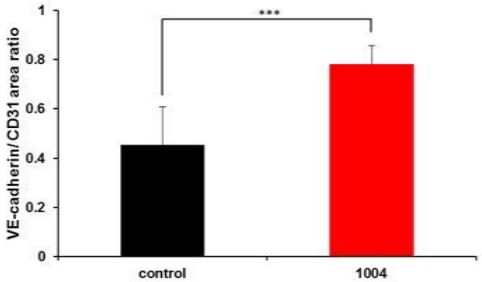

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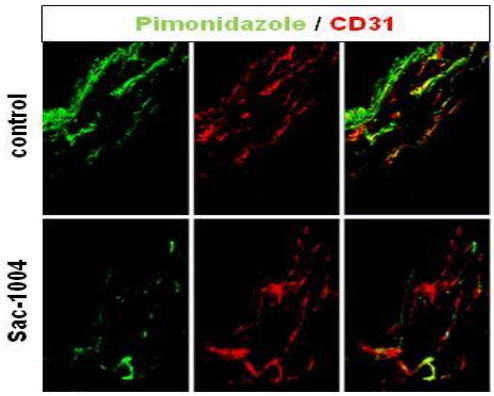

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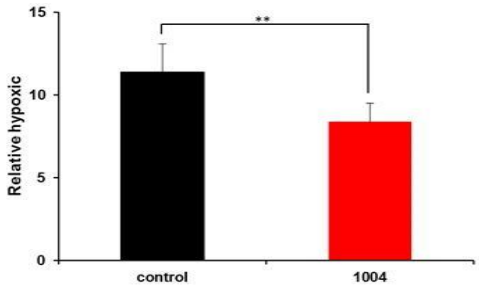


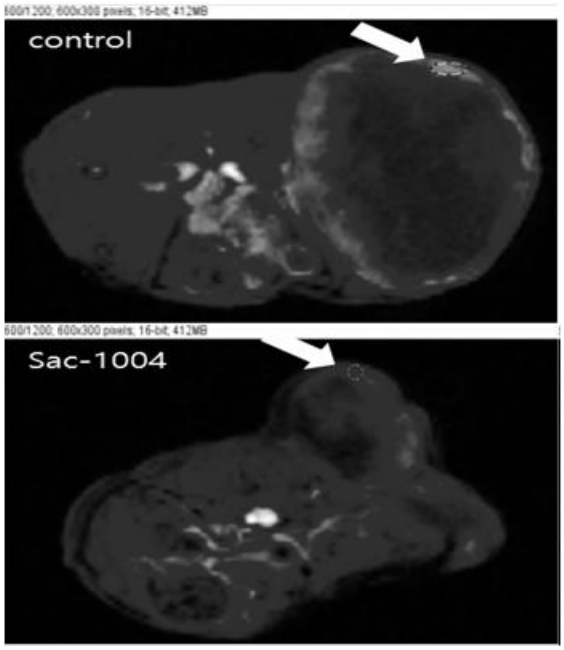
Figure 3. Treatment of Sac-1004 significantly increases VE-cadherin and decreases hypoxia in prostate cancer xenografts. A. Immunofluorescence staining of PC-3 tumor section (n=6), treated with Sac-1004 or control, for CD31 and VE-cadherin. Representative images from different tumors have been shown. Scale bar, 400 μm. B. Images shown in A. were quantified using ImageJ software. C. Immunohistochemical analysis of PC-3 tumors section (n=6) for CD31, pimonidazole in the peritumoral zone. Scale bar, 400 μm. D. Images shown in C. were quantified using ImageJ software. Data are represented as mean ± S.D; **P<0.01; ***P<0.001.

4. Effects of vascular normalization on vascular perfusion in prostate cancer

The results of the previous experiments indicate that sac-1004 normalizes vessel structure by reducing tumor vascular leakage. It means that normalized vessel was improved vessel structure and function. Therefore, we further evaluated the perfusion of the normalizing tumor vessels through dynamic contrast-enhanced magnetic resonance images (DCE-MRI). It can show the sensitivity to characteristics of vasculature such as vessel perfusion and permeability. The measured effect of contrast agent diffusion is shown in the white-circled area (ROI) of figure 4A.

Signal intensity of sac-1004 treatment group is certainly increased against the control group. Especially, signal intensity is increased to two times at 60s after contrast injection compared to the control group. This data indicate that that the perfusion of the contrast agent significant increase in the normalized vessels (Fig. 4B).

A



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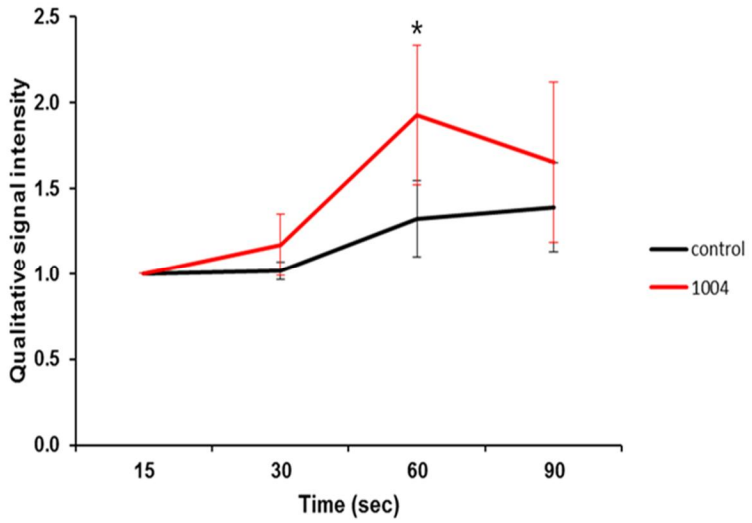


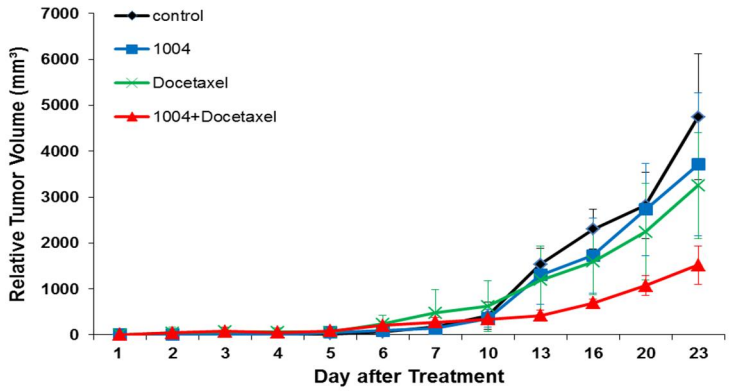
Figure 4. Sac-1004 improves vascular perfusion of tumor vessels in human prostate cancer xenografts. (A). Representative pictures for MR images of mice bearing PC-3 xenografts after sac-1004 or vehicle treatment (n=5). Signal intensity curve relies on qualitative analysis in the ROI (arrow). (B). Qualitative analysis of MR perfusion in PC-3 xenografts after 7-days intravenous injection of sac-1004 or vehicle (control).

5. Effect of vascular normalization on delivery of chemotherapy in prostate cancer

We showed that Sac-1004 was able to normalize the tumor vessels and reduced the hypoxic microenvironment and improved the vascular perfusion. Then, we postulated that vascular normalization enhances the accessibility of chemotherapeutic agent into tumor and results in efficacy of drug delivery. Docetaxel has been used as a standard chemotherapeutic agent for advanced prostate cancer. Combination of Sac-1004 and docetaxel indicated that tumor growth was not much affected by docetaxel alone, but the growth of PC-3 tumors was suppressed by combination treatment (Fig. 5A). Analysis of end-point tumor volume indicated the tumor volume was reduced in Sac-1004 with docetaxel group as compared to docetaxel or Sac-1004 treatment alone (Fig. 5B).

To evaluate an effect of vascular normalization on the efficacy of drug delivery in prostate cancer, we measured the intratumoral concentrations of docetaxel in PC-3 xenografts. Vascular normalization significantly increased intratumoral docetaxel concentration at the same plasma concentration, while had no effects on docetaxel concentrations in normal tissue (liver) (Fig. 5C). Thus, our data demonstrates that normalizing the tumor vasculature is a promising strategy to suppress tumor progression by enhancing efficacy of drug delivery in prostate cancer.

A



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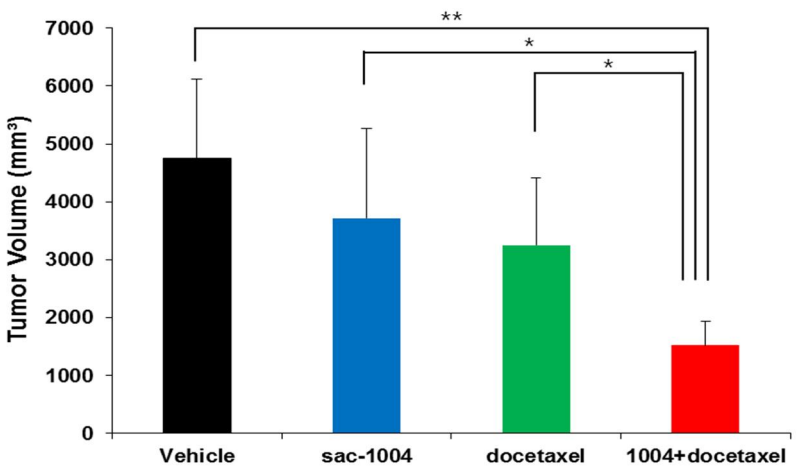

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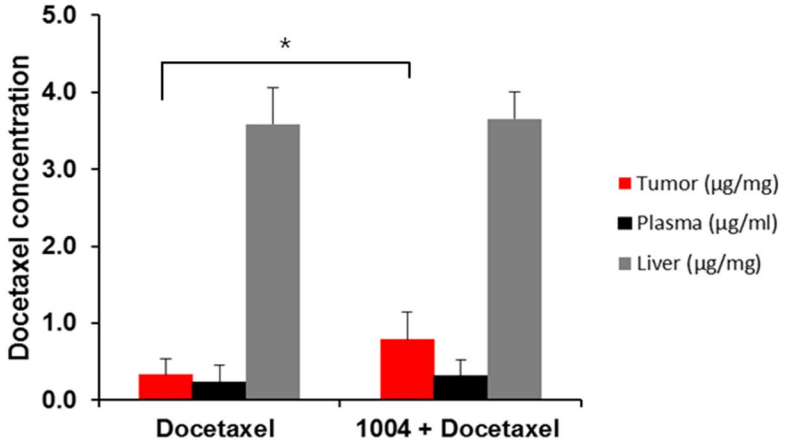

Figure 5. Sac-1004 enhances the anti-tumor activity of docetaxel in human prostate cancer animal model. (A). PC-3 xenogrfts were treated intravenously with sac-1004 or vehicle for 7 days and then randomized into two groups for further treatment with docetaxel or vehicle. Docetaxel was injected intravenously every week and tumor growth was monitored using measuring tumor size. (B). Relative tumor size at the end point of the in vivo study (n=4). (C). Docetaxel concentration in Tumor, plasma and tissue (n=6). Data are represented as mean ± S.D; *P<0.05 **P<0.01

IV. DISCUSSION

Abnormal vessels; leaky, dilated, saccular, and tortuous blood vessels are a hallmark of not only cancer but also of a number of pathological conditions like ischemia, inflammation.[1] Abnormal vasculature steadily aggravates the blood perfusion in tumors and as a result, hypoxic and acidosis tumor microenvironment makes the tumor more aggressive. Abnormal tumor microenvironment increase their invasive and metastatic potential, and apply selective survival pressures to which cancer cell populations adapt.[1] Furthermore, poor perfusion and leaky vasculature limit the delivery of chemotherapeutic agents to tumor and so make difficult chemo/radio therapy.[3]

Lots of molecules have been identified to recruit in blood vessels, but most studies have focused on vascular endothelial growth factor (VEGF) and its receptors. The majority of solid tumors overexpressed VEGF. Thus, if VEGF signaling in tumors was down-regulated, the vasculature might back to a normal condition.[16] These structural changes are accompanied by functional changes-decreased interstitial fluid pressure, increased tumor oxygenation, and improved efficacy of drugs in tumors.[17-20] VEGF targeting drugs, inducing antiangiogenic therapies, have been approved for cancer therapy. But, these inhibitors decreased both tumor growth and blood vessel density, may cause hypoxia and aggravate tumor progression and treatment resistance by blocking tumors' blood supply.[1,21]

Recently, a number of studies have reported the opposite strategies aimed at normalizing vasculature alleviating tumor hypoxia while improving perfusion may enhance the outcome of chemotherapy, and radiotherapy and immunotherapy. We also investigate about tumor vessels normalization. Sac-1004 is known to block vascular leakage by enhancing endothelial integrity via the cAMP/Rac/cortactin pathway.[10] Here, we confirmed how the endothelial leakage blocker, Sac-1004, can normalize tumor vessels and how normalizing vessel can affect therapeutic efficacy of chemotherapy in prostate cancer. We initially investigated the effect of leakage blocker on HUVECs and prostate cancer cells. Leakage blocker had effect only on HUVECs but had no effect on

tumor cells. In common with *in vitro*, leakage blocker wasn't able to reduce the tumor mass of PC-3 subcutaneous xenografts. Leakage blocker directly stabilized the tumor endothelial junction by upregulation of junction proteins such as vascular endothelial (VE)-cadherin. Directly reorganized of tumor endothelial junction was able to normalize blood vessels.

Normalized vasculature alleviated impaired blood perfusion in tumors and results in reduced hypoxic tumor microenvironment. Hypoxia also induces cancer stem cell phenotype and enables resistance to many use therapeutic agents.[1,22] Intratumoral hypoxia correlates with a poor prognosis in many human cancers. Besides, tumor vascular functionality plays a vital role in the efficiency of chemotherapy. These facts suggest that combination therapy with leakage blocker and docetaxel can control hypoxia development in tumor microenvironment and therapeutic effects may have better. Upon combination of docetaxel with leakage blocker, we found that combination therapy of docetaxel and leakage blocker was able to decrease tumor mass of PC-3 subcutaneous xenografts. In addition, drug delivery efficacy in combination therapy of docetaxel and leakage blocker was better than docetaxel monotherapy. To gain in bioavailability and selectivity toward tumor cells, therapeutic molecules must transfer to tumor cells from tumor vessels.[23] Tumor vasculature is an important determinant of the efficacy of drug delivery.

In conclusion, our study demonstrated that directly normalized vasculature can alleviate tumor microenvironment and found that combining leakage blocker with chemotheraphy improve the efficacy of drug delivery. This strategy for improving combination therapy outcomes can provide with clinical trials.

V. CONCLUSION

Leakage blocker improves hypoxic tumor microenvironment and restores abnormal leak vasculature of tumor by restoring endothelial cell junction in prostate cancer. Vascular normalization induced pairing blood perfusion and docetaxel combination following vascular normalization sensitized prostate cancer to docetaxel treatment.

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ABSTRACT(IN KOREA)

혈관 정상화가 전립선 암의 성장과 치료 효율성에 미치는 영향

<지도교수 홍 성 준>

연세대학교 대학원 의과학과

강 유 현

혈관생성 억제 치료는 종양의 혈관생성을 억제하여 종양의 성장에 필요한 산소와 영양분의 공급을 차단함으로써 종양의 진행을 억제한다. 그러나 혈관의 억제를 통해 유발되는 허혈 상태의 악화는 결과적으로 더욱 공격적인 형태의 암세포를 양상하게 되며 한편으로는 약물의 저항성을 증가시키게 된다. 종양 혈관은 정상 조직의 혈관과는 달리 비정상적인 형태를 보이며, 기능적으로 누출도가 높고 관류성이 낮아 결과적으로 pH가 낮고 간질내 압력이 증가하는 상태의 종양 미세 환경이 형성한다. 한편, 관류성의 저하는 종양 내로의 효율적인 약물 전달을 방해함으로써 항암제들의 치료 효과를 감소시키고 반대로 부적용을 증가시킬 수 있다. 본 연구에서는 비정상적인 종양 혈관 구조를 정상화시켰을 때, 전립선암의 성장과 약물 치료에 미치는 영향에 대하여 연구하였다. 혈관세포 안정화 약물인 SAC-1004를 이용하여 in vitro와 in vivo에서 혈관내피세포의 증식, 혈관 누출도 감소, 관류성 증가를 보여주었다. 또한, 혈관 정상화를 통해 종양내 허혈 상태가 개선됨이 HIF1a의 감소를 통해 확인되었으며, 동물모델에서 혈관의 정상화 이후 도세탁셀을 정주하였을 때, 정상 조직 대비 종양 내에서 도세탁셀의 농도를 의미 있게 증가시키며 더욱 효과적으로 암의 성장이 억제된다는 것을 확인하였다. 이러한 결과들을 통해 혈관 정상화 약물을 이용한 종양 혈관의

정상화가 항암제의 암 억제 효과를 극대화시키고 부작용을 줄일 수 있는 효과적인 방법이 될 수 있음을 제시하였다.

핵심되는 말 : 전립선 암, 혈관 정상화, 종양혈관형성, 도세탁셀